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Evidence that vasoactive intestinal polypeptide is a parasympathetic neurotransmitter in the endocrine pancreas in dogs

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Abstract

Vasoactive intestinal polypeptide (VIP) has been found in pancreatic nerves in several species. Studies were conducted to determine if VIP could be a parasympathetic neurotransmitter in the canine endocrine pancreas. To verify that VIP is localized in pancreatic parasympathetic nerves, sections of canine pancreas were immunostained for VIP. VIP staining was identified in the majority of neuronal cell bodies in intrapancreatic parasympathetic ganglia. In addition, VIP was localized in nerve fibers innervating pancreatic islets in the proximity of alpha cells. Next, to determine if VIP is released during electrical stimulation of parasympathetic nerves, pancreatic spillover of VIP was measured during vagal nerve stimulation (VNS) in anesthetized dogs. VIP spillover increased from a baseline of 630 ± 540 pg/min to 2580 ± 540 pg/min ($\Delta = +1950\pm490$ pg/min, $p < 0.01$). Pancreatic VIP release during VNS was not affected by atropine, whereas ganglionic blockade with hexamethonium nearly abolished the VIP response to VNS ($p < 0.005$ vs control), suggesting that VIP is a postganglionic neurotransmitter in the dog pancreas. To examine the effects of VIP on pancreatic hormone secretion, synthetic VIP was infused locally into the pancreatic artery. VIP, at a low dose (5 pmol/min), increased glucagon secretion from 1750 ± 599 to 3800 ± 990 pg/min ($\Delta = +2060\pm870$ pg/min, $p < 0.05$), but did not affect insulin secretion ($\Delta = -1030\pm760$ μU/min, NS). Thus, VIP is contained in and released from pancreatic parasympathetic nerves in proximity to islet alpha cells and exogenous VIP, at a dose which approximates the increase of VIP spillover during VNS, preferentially stimulates glucagon vs insulin secretion. Therefore, VIP is likely to function as a parasympathetic neurotransmitter in the endocrine pancreas in dogs. We hypothesize that VIP could mediate the glucagon response to parasympathetic activation which has been shown to resistant to cholinergic blockade with atropine in several species.

Keywords: Atropine; Hexamethonium; Acetylcholine; Glucagon; Insulin; Somatostatin

1. Introduction

Acetylcholine, via its action on muscarinic receptors, is considered to be the primary post-ganglionic neurotransmitter of the parasympathetic nervous system. However, there is also evidence for peptidergic mediation of a number of parasympathetic actions. For example, gastrin release during vagal activation is not entirely blocked by atropine, and a neuropeptide, gastrin releasing polypeptide, contributes to vagally-induced gastrin release [1]. Similarly, some of the pancreatic endocrine responses to vagal nerve stimulation are insensitive to muscarinic blockade by atropine. For example, we have found that atropine has no

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effect on the glucagon response to vagal nerve stimulation, but markedly reduces vagally-induced insulin secretion in dogs, whereas ganglionic blockade with hexamethonium abolishes both responses [2]. Similar findings have been reported in other species [3,4]. These findings suggest that one or more neuropeptides may be functioning as post-ganglionic parasympathetic neurotransmitters in the endocrine pancreas [5,6].

One such neuropeptide, vasoactive intestinal polypeptide (VIP) was among the first of a number of candidate neuropeptides found in pancreatic nerves [7]. VIP is present in pancreatic nerves in several species [7–11] including humans [8,12,13] and results from one study suggest that VIP is released from the pancreas and may contribute to the vagal stimulation of pancreatic exocrine secretion and blood flow in pigs [10].

In this study, we present evidence implicating VIP as a peptidergic neurotransmitter in the endocrine pancreas in dogs. To that end, we first verified the localization of VIP in pancreatic parasympathetic ganglia by immunostaining. We next examined pancreatic islets for the presence of VIP containing nerve fibers and the relationship of these nerves with islet alpha-cells by dual immunostaining. We then quantified the amount of VIP released from the canine pancreas during vagal nerve stimulation in anesthetized dogs and examined the effects of muscarinic blockade with atropine or nicotinic, ganglionic blockade with hexamethonium on vagally-induced pancreatic VIP release. Lastly, we examined the effects of a local infusion of synthetic VIP into the pancreatic artery on pancreatic hormone secretion in anesthetized dogs.

2. Materials and methods

2.1. Immunohistochemical studies

Pancreas tissue was harvested at necropsy from purpose-bred mongrel dogs of either sex. Small pieces of pancreas were fixed by overnight immersion in 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C, rinsed in 70% ethanol, and embedded in paraffin. Immunohistochemistry was performed on 5 μm sections by indirect immunofluorescence.

For VIP immunostaining, a monoclonal antibody to VIP (kindly provided by Dr. Alison Buchan, University of British Columbia, Vancouver, Canada) was used at a dilution of 1:20 000 (in 0.05 M PBS with 1% BSA) and visualized using Cy3-labeled anti-mouse lgG at 1:100 (Jackson Immunoresearch Laboratories, West Grove, PA). Sections were mounted in glycerol and viewed with a Nikon Microphot-FXA fluorescent microscope. For double-immunostaining of VIP and glucagon, sections were first incubated overnight with the VIP antibody followed by 1 h incubation in the presence of Cy3-labeled anti-mouse IgG. The sections were then incubated overnight with a polyclonal antiserum to glucagon (dilution 1:1000), followed by 1 h with fluorescein-labeled anti-rabbit IgG (1:100; Jackson) [14,15]. The double-immunostained sections were viewed with a Zeiss Axioplan fluorescent microscope equipped with a Hamamatsu C4880 fast-cooled integrating camera. Separate images were acquired using appropriate filters (rhodamine for VIP or fluorescein for glucagon), then they were pseudo-colored (red for VIP and green for glucagon) and fused into a single image using M2 software on the MCID image analysis system (Imaging Research, Brock University, St. Catharines, Ontario, Canada). Controls showed that no fluorescein fluorescence was visible when sections were viewed with rhodamine filters for Cy3, and Cy3 fluorescence was not detected when sections were viewed with FITC filters.

2.2. Animals and surgical preparations

Two types of experiments were performed. In one study, endogenous VIP release was measured before and during electrical vagal nerve stimulation. In the second study, pancreatic hormones and VIP levels were measured before and during infusion of exogenous VIP directly into the artery supplying the duodenal lobe of the pancreas. Mixed breed male dogs were fasted overnight prior to the procedures. Animals used in the experiments with exogenous VIP infusion were anesthetized with intravenous pentobarbital, sodium (30 mg/kg with a continuous infusion of 0.05 to 0.2 $mg \cdot kg^{-1} \cdot min^{1}$). Dogs were ventilated with room air (tidal volume = 20 ml/kg) with a respirator. Animals used in the nerve stimulation experiments were induced with intravenous injection of a short-acting barbiturate, thiamylal, sodium (20 mg/kg) and maintained on a respirator connected to a vaporizer (Draeger, Germany) supplying 0.8% halothane in oxygen. We have previously demonstrated that this anesthetic regimen allows larger pancreatic endocrine responses to electrical vagal nerve stimulation than does pentobarbital which suppresses both reflex vagal activation and nerve stimulation-induced pancreatic polypeptide secretion [16].

In both groups of dogs, a femoral artery (FA) was catheterized for blood sampling and blood pressure measurement. A femoral vein was catheterized for infusion of 0.9% saline and drugs. In all dogs, a midline laparotomy was performed and an extracorporeal shunt containing a sampling port, an electromagnetic flow probe (in Vivo Metric Systems, Healdsburg, CA), and a heparin infusion line was placed between the superior pancreaticoduodenal vein (SPDV), the major vessel draining the right lobe of the pancreas, and the portal vein. This preparation allows the measurement of pancreatic hormone output [17] and neurotransmitter spillover [18,19] from the right lobe of the pancreas. In dogs which received intrapancreatic infusions of VIP, the duodenum was surgically isolated from the duodenal lobe of the pancreas as previously described [18]. This procedure excludes the venous drain-

age from the duodenum from reaching the SPDV and effectively converts this vessel into a superior pancreatic vein (SPV). This procedure was not done in the vagal nerve stimulation experiment since we have shown that duodenal exclusion abolishes pancreatic endocrine responses to vagal nerve stimulation [20], presumably because the vagal nerve fibers innervating the pancreas traverse the duodenum.

In order to assess pancreatic VIP release during vagal nerve stimulation, the right and left branches of the cervical vagal nerve trunks were isolated from the fascia adjacent to the common carotid arteries [16]. The vagal trunks were cut between two ligatures and the distal segment of each was connected to a bipolar electrode (Harvard Apparatus, South Natick, MA). The experimental protocols were conducted after a 1-h stabilization period.

In dogs in which VIP was administered locally into the arterial blood supplying the right lobe of the pancreas, a 22 gauge Teflon catheter (Abbott, Inc., North Chicago, IL) was inserted into the superior pancreatic artery [18]. Adequate perfusion of the pancreas was verified at the conclusion of each experiment by infusing a dye, sodium indigotindisulfonate (Hynson, Wescott, and Dunning, Baltimore, MD), into the pancreatic arterial catheter.

2.3. Experimental protocols

To assess pancreatic VIP release during electrical vagal nerve stimulation, the vagal nerves were stimulated for 10 min with square-wave pulses of 5 ms duration and 13.5 mA current at a frequency of 10 Hz [15]. The stimulations were performed with a Grass model S-44 stimulator with a Grass PSIU6 constant current stimulus isolation unit (Grass Instruments, Quincy, MA). Paired blood samples were drawn from the FA and SPDV at -5 and 0 min before, at 5 and 10 min during, and at 5 and 15 min after the stimulation. Nerve stimulations were conducted under control conditions during saline infusion, during muscarinic receptor blockade with atropine sulfate (Eli Lilly, Indianapolis, IN) at a dose of 0.25 mg/mg + 0.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, or blockade of nicotinic ganglionic neurotransmission with hexamethonium bromide (Sigma Chemical, St Louis, MO) at a dose of 0.1 mg/kg + 0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The dose of atropine is sufficient to block the cholinergically-mediated increase of pancreatic polypeptide secretion during electrical vagal nerve stimulation in dogs [21]. The dose of hexamethonium is sufficient to eliminate all of the pancreatic endocrine response to vagal nerve stimulation in dogs [2,21].

To examine the effects of exogenous VIP on pancreatic hormone secretion, paired baseline blood samples were drawn from the FA and SPV at -10 and 0 min. Then synthetic VIP (Peninsula Laboratories, Belmont, CA) dissolved in saline was infused into the pancreatic artery at a dose of 5 pmol/min for 30 min at a rate of 0.1 ml/min. The sequence of VIP is identical in most mammals including humans, rats, pigs, and dogs. Paired blood samples were drawn at 5, 10, 15, 20, and 30 min during, and at 5, 15, and 30 min after the VIP infusion was discontinued. In all studies blood flow in the SPDV/SPV circuit was measured with an electromagnetic flowmeter, (Model SV4F-4RD, Zepeda Instruments, Seattle, WA). Hematocrit was determined at regular intervals throughout the experiments.

2.4. Assays

Blood samples for glucose and insulin determination were drawn and placed in tubes containing EDTA, for glucagon determination in tubes containing heparin and benzamidine HCl, and for somatostatin and VIP determination in tubes containing a solution of anticoagulants and proteolytic enzyme inhibitors [22]. All samples were kept on ice until centrifugation (20 min at 4°C). The plasma was separated and frozen at -20°C until assayed.

Plasma glucose was assayed by the glucose oxidase method with a Technicon Autoanalyzer (Technicon Instruments, San Francisco, CA). Plasma immunoreactive glucagon (IRG) was measured by radioimmunoassay (RIA) in unextracted plasma with an antibody that has high specificity for the C-terminal portion of the glucagon molecule [23]. Plasma immunoreactive insulin was measured by RIA with a modification of the double antibody technique of Morgan and Lazarow [24]. Plasma somatostatin-like immunoreactivity was measured in unextracted plasma with an assay using [^{125}I- Tyr11]somatostatin tracer and an antibody (1:105 000 final dilution) which is directed against the central portion of the somatostatin-14 molecule [25]. Plasma VIP was measured with a radioimmunoassay using an ^{125}I-VIP tracer, a rabbit anti-VIP sera (Calbiochem-Behring, La Jolla, CA,), and a sheep anti-rabbit 2nd antibody [26].

2.5. Calculations and data analysis

Pancreatic insulin, glucagon and somatostatin output during VIP infusion was calculated as: $[\text{Hormone}]_{SPV} - [\text{Hormone}]_{FA} \times$ Blood Flow $\in$ the SPV $\times$ (1 − hematocrit). The changes of pancreatic hormone output were calculated by subtracting the mean of the -10 and 0 minute baseline values from the mean of the 10, 15, 20, and 30 minute values during the infusion of VIP. The changes of VIP concentrations and pancreatic VIP output during vagal nerve stimulation were calculated by subtracting the mean of the -5 and 0 minute baseline values from the mean of the 5- and 10-min values during nerve stimulation. Pancreatic VIP output during VIP infusion or neuronal VIP spillover during vagal nerve stimulation was calculated as: $[\text{VIP}]_{SP(D)V} - [\text{VIP}]_{FA} \times$ SP(D)V Blood Flow $\times$ (1 − hematocrit). This calculation slightly underestimates pancreatic VIP release during vagal nerve stimulation because the pancreas extracts approximately 50% of

VIP arriving in the arterial blood (unpublished observation) as it does the classical sympathetic neurotransmitter, norepinephrine [27] and other neuropeptides such as galanin [28].

The data are expressed as mean ± SE. Statistical comparisons of means within a group were made with a paired *t*-test. For comparisons between groups, analysis of variance was performed with a Dunnett's post-test. *P* values less than 0.05 were considered significant.

3. Results

3.1. VIP immunoreactive nerves in canine pancreas

To verify that canine pancreatic VIP-containing neurons are parasympathetic, we immunostained sections of dog pancreas for the presence of VIP immunoreactivity in intrapancreatic ganglia, since these ganglia contain the neuronal cell bodies which give rise to the post-ganglionic, parasympathetic nerve fibers in the pancreas [29]. In every intrapancreatic ganglion examined, VIP was observed in the majority of neuronal cell bodies (Fig. 1 A and B and Table 1). Small nerve trunks containing VIP-positive fibers were sometimes observed emanating from intrapancreatic ganglia (Fig. 1 B).

To determine whether these intrinsic VIP-containing nerve fibers project to alpha cells in islets of the canine pancreas, we performed double-immunostaining of sections of dog pancreas for VIP and glucagon. The results revealed numerous VIP-immunopositive nerve fibers innervating pancreatic islets (Fig. 2A). Many of these fibers had a beaded appearance; these varicosities are presumably sites of 'en passant' release of VIP [30]. Such VIP-containing varicosities were often found in close proximity to glucagon-immunoreactive cells within islets (Fig. 2B).

3.2. Effects of vagal nerve stimulation (VNS) on pancreatic VIP release

Electrical stimulation of the cervical vagal trunks produced a small increase of arterial VIP, a quadrupling of pancreatic venous VIP concentrations with little effect on pancreatic vein blood flow. leading to a 3-fold increase of pancreatic VIP spillover (Table 2).

Arterial VIP, pancreatic venous VIP, and VIP spillover responses during atropine administration were similar to those during the control saline infusions. The increase of pancreatic venous VIP concentrations during BCVNS was markedly reduced by hexamethonium and the increment of VIP spillover was inhibited by more than 90% (Fig. 3 Table 2).

3.3. Effects of intra-pancreatic arterial (PA) infusion of exogenous VIP

Infusion of a low dose of VIP (5 pmol/min) directly into the pancreatic artery did not increase systemic (femoral) arterial VIP levels (data not shown). However, pancreatic venous VIP levels increased significantly and pancreatic vein blood flow increased by 81±33% during VIP infusion, leading to a marked increase of pancreatic VIP output from 410±80 to 5200±840 pg/min ($\Delta = +4780 \pm 870$ pg/min, $p < 0.0025$, Fig. 4A).

Pancreatic arterial infusion of this dose of VIP more than doubled pancreatic glucagon output from 1750±590 to 3800±990 pg/min ($\Delta = +2050 \pm 870$ pg/min; +225± 105%, $p < 0.025$, Fig. 4B). In contrast, VIP did not sig-

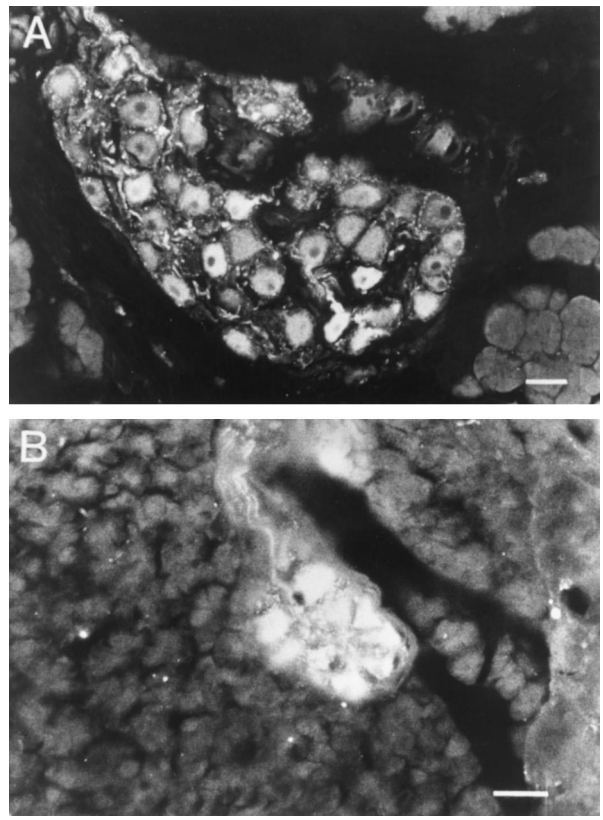

Fig. 1. Presence of VIP immunoreactivity in neuronal cell bodies in dog intrapancreatic ganglia. (A) Section of dog pancreas immunostained for VIP, showing a large intrapancreatic ganglion with approximately 40 cell bodies. Approximately half of the neuronal cell bodies have VIP immunofluorescence, whereas in half of the cell bodies VIP immunofluorescence appears either less intense or absent. (B) A separate section of dog pancreas showing an intrapancreatic ganglion with 7–8 neuronal cell bodies, all with intense VIP immunofluorescence. Note nerve trunk containing numerous fibers with VIP-immunoreactivity which appears to exit ganglion. (in both figures, Bar = 50 μm).

Table 1
Proportion of neuronal cell bodies in large intrapancreatic ganglia exhibiting VIP immunofluorescence in five sections of pancreas from four dogs

Intensity of fluorescence	−	+	+ +	+ + +	Total
No. of cell bodies	2±1	6±2	10±2	9±2	26±4
% of cell bodies	7±4	21±5	35±6	37±11	100

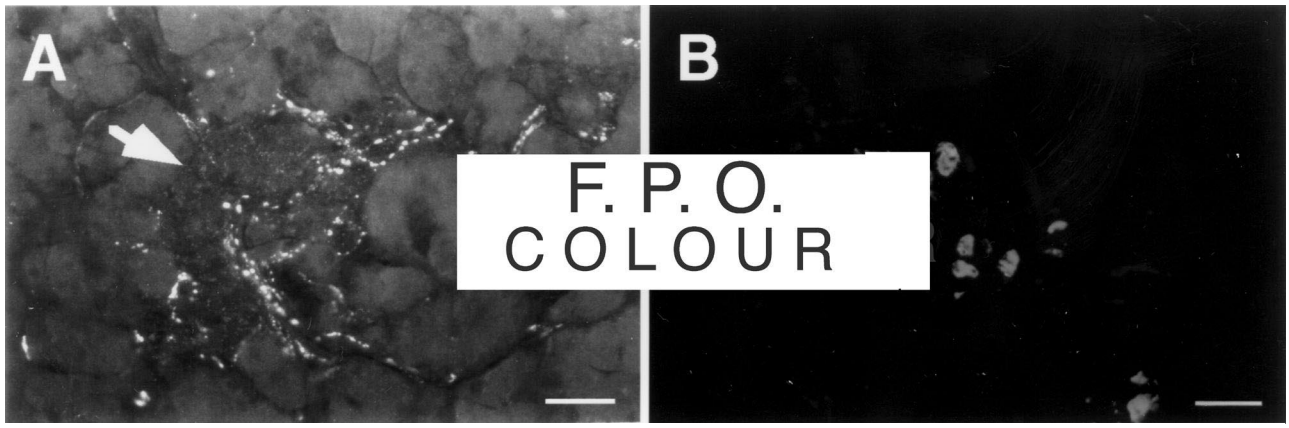

Fig. 2. Innervation of dog pancreatic islets by VIP-containing nerve fibers. (A) Section of dog pancreas immunostained for VIP. Note beaded appearance of VIP-immunopositive nerve fibers. (B) Section of dog pancreas was double-immunostained for the presence of VIP (red) and glucagon (green). The VIP and glucagon immunostains were acquired separately using appropriate filters, pseudocolored red (VIP) or green (glucagon), and the two images fused to produce the final figure as described in Section 2. Note presence of VIP-immunopositive nerve fibers in close proximity to glucagon-immunopositive cells. (in both figures, Bar = 25 μm).

Table 2
Plasma vasoactive intestinal polypeptide (VIP) concentrations and pancreatic VIP output (spillover from pancreatic parasympathetic nerves) before and during vagal nerve stimulation (VNS).

Treatment	VIP_{FA} (pg/ml)	VIP_{SPDV} (pg/ml)	BF_{SPDV} (ml/min)	VIP-output (pg/min)
BCVNS (control)				
Baseline	33±6	126±53	9.1±1.0	630±290
During VNS	88±30	505±89	9.7±0.8	2580±540
Δ	+54±28*	+379±68****	+0.7±0.8	1950±490***
BCVNS (atropine)				
Baseline	174±127	251±168	8.1±1.1	380±150
During VNS	254±173	557±256	9.2±1.3	1970±440
Δ	+80±47	+306±93**	+1.1±0.6	+1590±360***
BCVNS (hexamethonium)				
Baseline	26±5	43±13	6.6±1.0	70±40
During VNS	36±15	93±26	6.2±1.1	220±80
Δ	+10±9	+50±19**	−0.4±0.5	+150±70* ****

$n = 6$ per treatment; values are means±SE.
FA = Femoral Artery; SPDV = superior pancreaticoduodenal vein.
* = $p < 0.05$; ** = $p < 0.025$; *** = $p < 0.01$ vs baseline; **** = $p < 0.005$ vs control.

nificantly affect pancreatic insulin output which averaged 5460±2540 μU/min before and 4410±1910 μU/min during VIP infusion (Δ = 1030±760 μU/min; −2±11%, NS, Fig. 4C). Pancreatic somatostatin output increased during VIP infusion from 1150±210 to 1500±210 fmol/min (Δ = +350±170 fmol/min; +45±21%, $p < 0.05$, data not shown). Plasma glucose was unchanged during VIP infusion averaging 112±8 mg/dl prior to infusion and 111±6 mg/dl during the infusion.

A ten-fold higher dose of VIP (50 pmol/min) infused into the pancreatic artery produced a similar increase of glucagon output (Δ = +173±74%, $p < 0.05$), still no significant change of insulin output (Δ = +26±32%, NS), and larger increases of pancreatic somatostatin output (Δ = +119±43%, $p < 0.02$) and pancreatic venous blood flow (Δ = +295±158%, $p < 0.01$) (data not shown).

4. Discussion

In the present study, immunostaining of sections of dog pancreas revealed that the majority of cell bodies in intrapancreatic ganglia contain VIP. Since these ganglia are the source of the parasympathetic, post-ganglionic nerves which innervate the pancreas [29], our data suggest that the VIPergic nerves innervating the canine pancreatic islet are intrinsic nerves of parasympathetic origin. This hypothesis is supported by other studies which demonstrated the presence of VIP immunoreactivity in neuronal cell bodies of intrapancreatic ganglia [7,11] and studies demonstrating that extrinsic denervation of the canine pancreas did not reduce the number of VIP containing nerves in the islet [9,11]. Further, we have observed that the majority of neuronal cell bodies in dog celiac ganglion, which supply

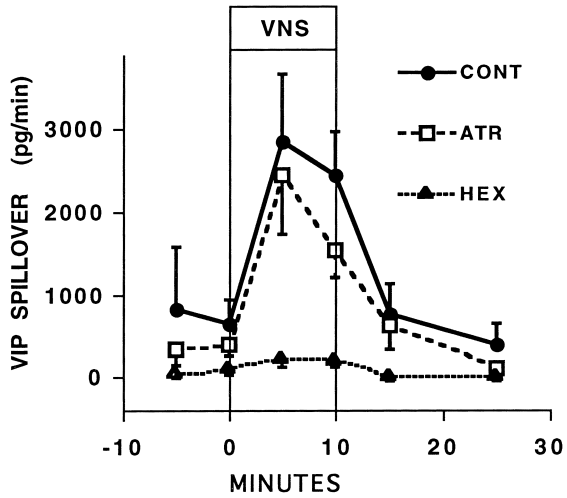

Fig. 3. Pancreatic VIP output (spillover) before, during, and after vagal nerve stimulation (VNS) in halothane-anesthetized dogs under control (CONT) conditions, with administration of atropine (ATR), or with administration of hexamethonium (HEX)($n = 6$ stimulations each).

the sympathetic post-ganglionic fibers that innervate the islet [29], do not contain detectable VIP immunoreactivity (unpublished observations), demonstrating that the VIP-ergic nerve fibers innervating dog islets are not of sympathetic origin.

The finding of VIP-containing neurons in the proximity of pancreatic A-Cells provides morphologic support for our hypothesis that VIP release from pancreatic nerves regulates islet hormone secretion in the dog. These fibers have the classical beaded appearance of peripheral autonomic nerves (Fig. 3). The beads (or varicosities) have been interpreted as multigranular release sites along the length of these highly branched fibers [30]. Only rarely do these beaded fibers appear to be in direct contact with A-Cells, rather they appear to pass in close proximity to A-cells (Fig. 3), presumably releasing VIP 'en passant'. This observation is consistent with that described for the autonomic innervation of other tissues which also have few direct synaptic contacts with autonomic nerves. Rather, the target tissues appear to be exposed to neurotransmitters that diffuse over a distances seven to one hundred times greater than those of a true synaptic cleft [31,32].

VIPergic innervation of the pancreatic islet has also been reported in other species, including humans [8,12,13], rats [8], and pigs [10], although these studies did not examine the specific innervation of A-cells. However, in humans, VIP-positive nerve fibers were reported to form a mesh around the islet periphery [8], where A-cells are most abundant. These similarities in the relationship of VIPergic nerves to A-cells in dogs and humans suggest that the dog may be a good model for performing mechanistic studies on the role of VIP in vagal regulation of the A-cell that cannot be performed in humans.

We also report for the first time that VIP is released from the canine pancreas during vagal nerve stimulation.

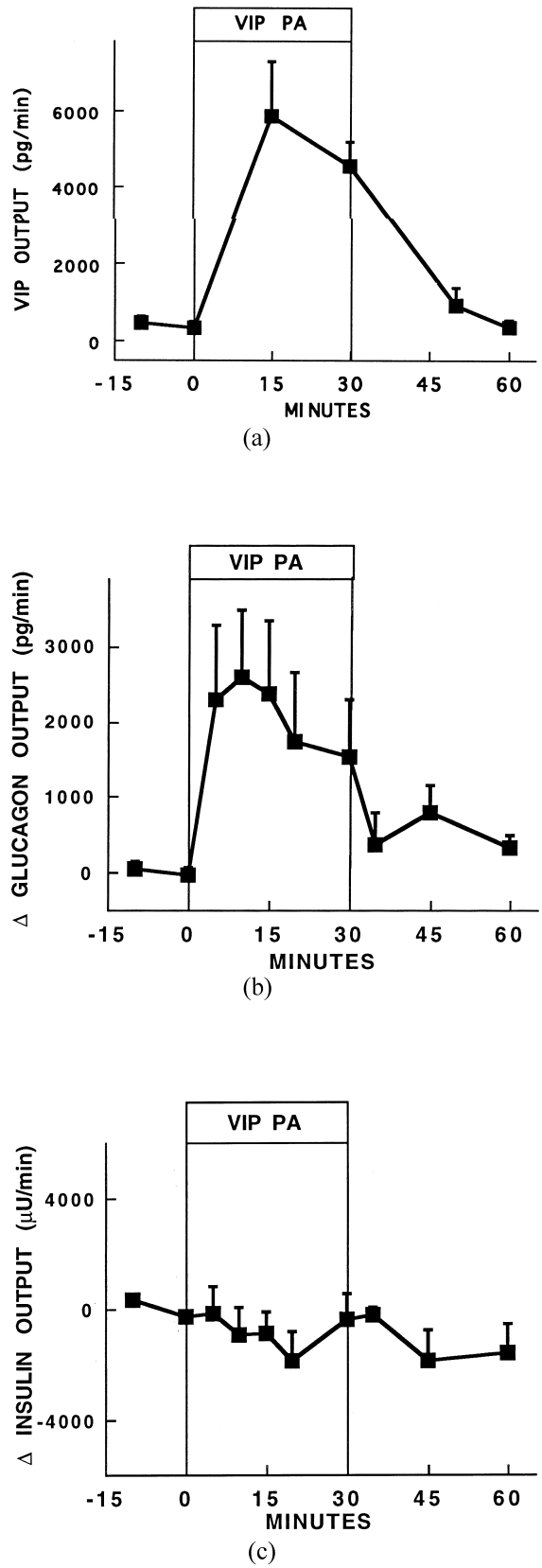

Fig. 4. (A) Pancreatic VIP output; (B) the change (A) of pancreatic glucagon output, and; (C), insulin output before, during, and after the infusion of synthetic VIP (5 pmol/min) into the superior pancreatic artery (PA) of seven halothane-anesthetized dogs.

The magnitude of the pancreatic VIP spillover during vagal nerve stimulation is similar to the VIP output measured during the low dose (5 pmol/min) intrapancreatic VIP infusion that stimulated glucagon secretion. Although the neurally released VIP is not concentrated in a true synaptic cleft (see above), the concentrations of VIP reaching the A-cell are likely to be significantly higher during vagal nerve stimulation than during intrapancreatic infusion of VIP.

Pretreatment with the muscarinic antagonist, atropine, did not affect pancreatic VIP release during vagal nerve stimulation. In contrast, ganglionic blockade with hexamethonium abolished pancreatic VIP release during vagal nerve stimulation. Our laboratory has previously reported that the glucagon response to electrical stimulation of the vagus nerves in dogs is unaffected by muscarinic blockade with atropine, whereas atropine markedly attenuates the insulin response to vagal nerve stimulation [2]. In contrast, ganglionic nicotinic receptor blockade with hexamethonium abolished both the glucagon and insulin responses to vagal stimulation [2]. The glucagon response to vagal nerve stimulation is similarly atropine-resistant in pigs [3] and rats [4], but not in calves [33]. These results suggest that in several species glucagon secretion can be stimulated by a noncholinergic neurotransmitter released from postganglionic parasympathetic nerves in the endocrine pancreas. However, to determine definitively whether VIP is in fact the mediator of the atropine-resistant glucagon response to vagal nerve stimulation will require experiments utilizing effective and selective antagonists of VIP action on the A-cell.

We found a preferential effect of VIP to increase glucagon secretion relative to insulin secretion during the infusion of low doses of VIP directly into the arterial supply of the pancreas. Similar findings have been reported in studies utilizing an in vitro porcine pancreas preparation [34]. In contrast, other studies have shown that VIP can increase both insulin and glucagon secretion [12,35]. This discrepancy may be due to the dose of VIP tested, the species studied, or the prevailing glucose level since the insulin response to VIP is potentiated by hyperglycemia [34]. Nonetheless, the results of the present study demonstrate that, in the dog, at euglycemia, the A-cell is more sensitive to VIP than the B-Cell.

In summary, we have introduced several lines of evidence that VIP functions as a peptidergic neurotransmitter in the endocrine pancreas in dogs: (1) VIP is contained in parasympathetic nerves in the canine pancreas; (2) These nerves innervate the islet in the proximity of A-cells; (3) Local infusion of low doses of VIP preferentially stimulate glucagon secretion; (4) VIP is released from the canine pancreas during vagal nerve stimulation in quantities sufficient to stimulate glucagon secretion. These data are consistent with the hypothesis that VIP could contribute to increased glucagon secretion during the activation of the parasympathetic nervous system. Furthermore, it is possible that VIP could help to mediate the increase of glucagon secretion during insulin-induced hypoglycemia since the pancreatic parasympathetic nerves are known to be activated during hypoglycemia [36,37]. Parasympathetic activation, along with sympathoadrenal activation, is a component of the redundant autonomic response which contributes to increased glucagon secretion during hypoglycemia [38–42].

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